SUMMARY

Emerging pathogens in honey bees: *Lotmaria passim*and *Crithidia mellificae*. Prevalence estimation and impact assessment on honey bee health in Italy

Key words: *Lotmaria passim, Crithidia mellificae,* tripanosomatids, prevalence, colony loss

Introduction

In recent years,honey bee colonies suffered from a general depopulation all over the world. In the United States,this phenomenon was represented by "colony collapse disorder -CCD", a syndrome characterized by the sudden disappearance from the hives, without any apparent cause, of most of the worker bees in the presence of the queen and immature brood in the hive. In Europe, although CCD has not been reported, the honey bee colonies have undergone a progressive decrease.

Recently, the attention of the scientific world has focused on emergingpathogens -trypanosomatids - possibly involved in honey bee colony losses. Until 2015, *C. mellificae*was the only known trypanosomatidof *Apis mellifera*; in that year Schwarz et al. characterized a new species, named *Lotmaria passim*, which today appears to be prevalent worldwide.

No comprehensive survey on *L . passim*and *C.mellificae*has been carried out in Italy until now and their prevalence and impact on honey bee health is still unknown.

The aimof this project is to estimate the presence and the prevalence of *C. mellificae*and *L. passim*in managed honey beesin Italy and to evaluate the possible association of these parasites, also in concomitance with other patogens, with honey bee mortality,

Specific aims are:

1. To check for the presence and to estimate the level of occurrence of trypanosomatids in Italian honey bees;
2. to isolate and genotypethe strains circulating in Italy;
3. to evaluate the possible association of these parasiteswith colony losses.

Materials and methods

A retrospective study was carried out on archives DNA collected from honey bee apiaries locatedin different italian regions. Samples were tested for the presence of *C.mellificae*and *L.passim*DNA by a PCR assay targeting cytochrome b.Honey bee gut samples were collected from PCR-positive apiaries to attempt thecultureisolation and genotyping of circulating strains by Whole-genome sequencing (WGS).

Results

The “herd”prevalence of *L. passim*was 74.0% in the first year and 41.4% in the second year. The“herd”prevalence of *C. mellificae*was 6.5%. The “within-herd”prevalence was from 30% to 60% and resulted quite homogeneous in the investigated italian regions. The “within-herd”prevalence of Pagina 6 di 27

*C. mellificae*in the few positive apiaries detected only in Trentino, Veneto and Sicily was from a minimum of 25% to a maximum of 100% with an average of 50%.
Prevalence data on trypanosomatids are being analyzed to evaluate statistically significant associations with mortality/depopulation,considering also the presence of other pathogens. The samples tested positive for *L. passim*by PCR in this study resulted 99-100% identical to *L. passim*Acc. No. MG494247 isolated in 2018 in USA. Samples positive for *C. mellificae*are under sequencing to confirm PCR results.
Twofield strains of trypanosomatids were isolated from 2 PCR-positive apiaries in the Lazio region, but bioinformatic analysis doesnot allow their identification since it was not possible to find a match with the databases used.

Discussion

This research project was the first national scale study carried out on trypanosomatids in managed italian honey bees. Ourresults show that honey bee trypanosomatids are present and confirm, in line with studies carried out in other European countries, that *L. passim*is the prevalentspeciesand*C. mellificae*seems to be sporadically present.
Regarding the characterization of the field strains of *L. passim*and *C. mellificae*circulating in Italy, another aim of this project, our field isolates did not match with the species present in the available databases.
In the next future we aim also to develop amplicon sequencing protocols for the identification of honey bee tripanosomatids through the sequencing of specific targets such as ITS, citB or alternative targets that may be more discriminating. The application of amplicon sequencing using HTS technology would allow us to analyze the large number of positive samples already collected and stored in our laboratory

.